

# Separation and Characterization of Four Hexose Kinases from Developing Maize Kernels

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## ABSTRACT

Four forms of hexose kinase activity from developing maize (*Zea mays* L.) kernels have been separated by ammonium sulfate precipitation, gel filtration chromatography, blue-agarose chromatography, and ion exchange chromatography. Two of these hexose kinases utilized D-glucose most effectively and are classified as glucokinases (EC 2.7.1.2). The other two hexose kinases utilized only D-fructose and are classified as fructokinases (EC 2.7.1.4). All hexose kinases analyzed had broad pH optima between 7.5 and 9.5 with optimal activity at pH 8.5. The two glucokinases differed in substrate affinities. One form had low  $K_m$  values [ $K_m(\text{glucose}) = 117$  micromolar,  $K_m(\text{ATP}) = 66$  micromolar] whereas the other form had much higher  $K_m$  values [ $K_m(\text{glucose}) = 750$  micromolar,  $K_m(\text{ATP}) = 182$  micromolar]. Both fructokinases had similar substrate saturation responses. The  $K_m(\text{fructose})$  was about 130 micromolar and the  $K_m(\text{ATP})$  was about 700 micromolar. Both exhibited uncompetitive substrate inhibition by fructose [ $K_i(\text{fructose}) = 1.40$  to  $2.00$  millimolar]. ADP inhibited all four hexose kinase activities, whereas sugar phosphates had little effect on their activities. The data suggest that substrate concentrations are an important factor controlling hexose kinase activity *in situ*.

The catabolism of sucrose in developing maize (*Zea mays* L.) kernels results in the production of glucose and fructose if catalyzed by invertase or of fructose and UDP-Glc if catalyzed by sucrose synthase in the presence of UDP. Free hexoses produced by either reaction must be phosphorylated before they can be further metabolized, although fructose may be converted to sorbitol by a ketose reductase found in maize endosperm (9). The phosphorylation of hexoses is catalyzed by any of several enzymes referred to collectively here as hexose kinases (HK<sup>1</sup>).

HK activity has been described in maize kernels previously. Cox and Dickinson (6, 7), working with partially purified HK from developing maize endosperm and embryos, measured some kinetic properties of fructose and glucose phosphorylating activity in these tissues and suggested that multiple forms of these enzymes might exist. Tsai *et al.* (16) measured GK activity in developing maize endosperm and found that this activity was much lower than activities of other carbohydrate metabolizing enzymes. Doehlert (9) measured ATP-dependent GK and FK activities as well as UTP-dependent FK

activity in developing maize endosperm and also found that the activities of these enzymes were 40- to 100-fold lower than either ketose reductase or sucrose synthase. The possibility that these enzymes may limit sucrose utilization in developing maize kernels was suggested. More recently, Doehlert *et al.* (10) examined the distribution of HK activities in developing corn kernels. Whereas most of the HK activity was found in the endosperm, the embryo contained higher HK activity relative to the endosperm on a dry weight basis. The activities of HK were also much lower than activities of other enzymes of carbohydrate metabolism measured.

The phosphorylation of hexoses by HK is essentially an irreversible metabolic step and represents a likely point of metabolic regulation. Relative to animal systems, much less is known about the role of HK in plant systems. In a metabolic study of germinating spring barley (12), it was found that metabolite levels for the HK and phosphofructokinase reactions deviated by 5 to 6 orders of magnitude from equilibrium levels, and these enzyme activities were lower than other glycolytic enzymes. It was suggested that these enzymes may function as pacemakers of glycolysis in germinating barley.

The objectives of this study were to separate different forms of HK chromatographically and to characterize their catalytic properties in order to gain insight into the regulation of hexose utilization by developing corn kernels.

## MATERIALS AND METHODS

### Plant Material

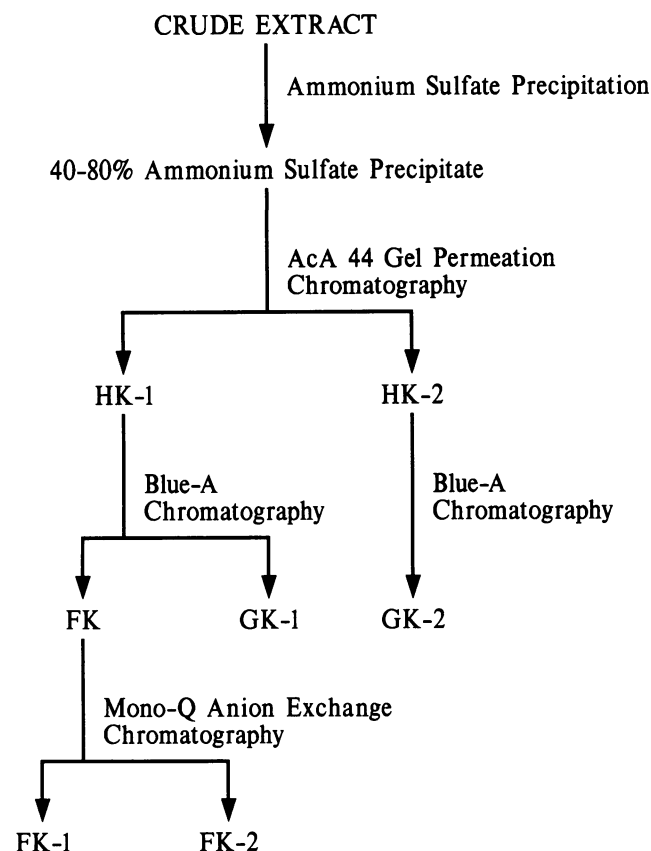
Corn (*Zea mays* L. inbred OH43) was grown in the greenhouse during the winter of 1986 to 1987. Plants were grown in 5 L pots containing Redi-earth<sup>2</sup> (Grace Corp., Cambridge, MA) supplemented with 60 g/kg Osmocote and 10 g/kg Micro-max (Sierra Chemical, Milpitas, CA). Supplemental light was provided by halogen vapor lamps for 12 h/d. Temperature was 28°C in the days and 22°C at night. Plants were fertilized weekly with soluble 20:20:20 fertilizer (Peters) supplemented with iron chelate. Ears were pollinated by hand and harvested 20 d after pollination. Kernels were stripped from ears, frozen immediately, and lyophilized. Whole lyophilized kernels were ground to a powder and stored at -90°C until enzyme extraction.

<sup>2</sup> The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

<sup>1</sup> Abbreviations: HK, hexose kinase; FK, fructokinase; GK, glucokinase.

### Enzyme Fraction and Purification

A flowchart illustrating the procedures used to separate four forms of HK from maize kernel extracts is shown in Figure 1. All purification procedures were carried out at 4°C. HK activity was extracted from whole kernel meal by homogenizing meal in 20 mL extraction buffer/g meal. Extraction buffer contained 50 mM Bicine-NaOH (pH 8.5), 5 mM MgCl<sub>2</sub>, and 1 mM DTT. Crude homogenates were centrifuged at 14,000g and the pellets were discarded. The supernatant was brought to 40% saturation with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, centrifuged as before, and the pellet was discarded. The resulting supernatant was then brought to 80% saturation with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and centrifuged. The supernatant was discarded, and the pellet was resuspended in 10 mM Bicine-NaOH (pH 8.5), 5 mM MgCl<sub>2</sub>, and 1 mM DTT. This preparation was then loaded onto a 2 × 120 cm Ultrogel AcA 44 column (LKB, Bromma, Sweden) equilibrated and eluted with 10 mM Bicine-NaOH (pH 8.5), 5 mM MgCl<sub>2</sub>, and 1 mM DTT. Five mL fractions were collected and each was assayed for GK and FK activity. Samples from Ultrogel fractionation were then loaded onto a 1.2 × 10 cm blue-agarose column (Amicon) that had been equilibrated with 10 mM Bicine-NaOH (pH 8.5), 5 mM MgCl<sub>2</sub>, and 1 mM DTT. The column was first washed with equilibration buffer while collecting 2 mL fractions, then washed with 10 mL 1 mM NAD in equilibration buffer, followed by 1 M NaCl in equilibration buffer. Fractions were assayed for FK and GK activity.



**Figure 1.** Flowchart illustrates the procedures used to separate four forms of HK from whole maize kernel extracts.

The FK preparations recovered from the blue-agarose column were applied to a 0.5 × 5 cm Mono Q anion exchange column connected with an automated liquid chromatography system (Pharmacia, Piscataway, NJ) equilibrated with 10 mM Bicine-NaOH, 5 mM MgCl<sub>2</sub> and 1 mM DTT. The column was eluted with a 0 to 300 mM NaCl gradient in the same buffer.

Chromatography of crude kernel soluble extract on Mono Q was performed as described above, except 10 mL of crude kernel extract was loaded onto the column. The enzyme extract was filtered successively with 3 μm, 0.45 μm, and 0.22 μm filters (Millipore) prior to loading onto the column. Conductivity was measured with a YSI conductivity meter (Yellow Springs, OH).

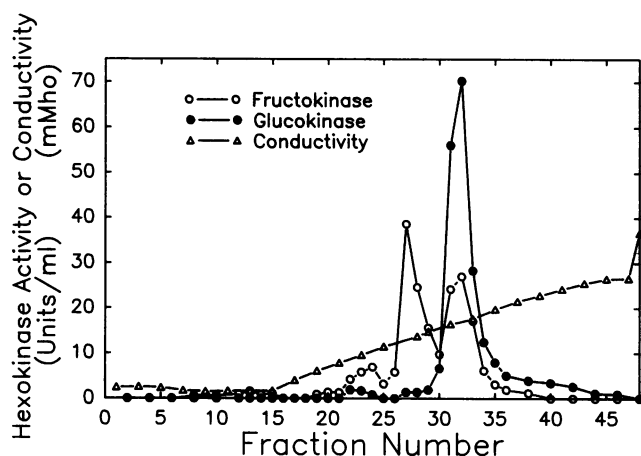
Analytical gel filtration was performed on a 1 × 60 cm Ultrogel AcA 44 column that was equilibrated and eluted with 10 mM Bicine-NaOH (pH 8.5), 5 mM MgCl<sub>2</sub>, and 1 mM DTT. Standard proteins used for *M<sub>r</sub>* calibrations were BSA (66,000), ovalbumin (44,000), pepsin (35,000), carbonic anhydrase (29,000), and chymotrypsinogen A (24,500).

### Enzyme Assays

HK activity was measured routinely by a continuous spectrophotometric assay that coupled hexose-phosphate production with glucose-6-P dehydrogenase and NAD reduction, by measuring the increase in *A*<sub>340</sub> (18). GK assays routinely contained 50 mM Bicine-NaOH (pH 8.5), 5 mM MgCl<sub>2</sub>, 5 mM glucose, 2.5 mM ATP, 1 mM NAD, and 1 IU/mL glucose 6-P dehydrogenase (from *Leuconostoc mesenteroides*, Sigma Chemical, No. G-5885). FK assays contained 50 mM Bicine-NaOH (pH 8.5), 5 mM MgCl<sub>2</sub>, 0.5 mM fructose, 2.5 mM ATP, 1 mM NAD, 1 IU/ml glucose 6-P dehydrogenase, and 1 IU/mL phosphoglucosomerase. Assays were initiated with hexose. Assays minus hexose were used as blanks. Activities were derived from the increase in *A*<sub>340</sub> as NAD was reduced.

HK activity was also measured by means of a continuous spectrophotometric assay that coupled ADP production with pyruvate kinase, lactate dehydrogenase and NADH oxidation, by measuring the decrease in *A*<sub>340</sub> (18). GK assays contained 50 mM Bicine-NaOH (pH 8.5), 5 mM MgCl<sub>2</sub>, 5 mM glucose, 2.5 mM ATP, 3 mM phosphoenolpyruvate, 0.3 mM NADH, 1 IU/ml pyruvate kinase, and 1 IU/ml lactate dehydrogenase. FK assays contained 50 mM Bicine-NaOH, 5 mM MgCl<sub>2</sub>, 0.5 mM fructose, 2.5 mM ATP, 3 mM phosphoenolpyruvate, 0.3 mM NADH, 1 IU/mL pyruvate kinase, and 1 IU/mL lactate dehydrogenase. Assays were initiated by adding hexose. Blanks contained no hexose. Activities were derived from the decrease in *A*<sub>340</sub> as NADH was oxidized. All continuous spectrophotometric assays were performed with a final volume of 1 mL at 30°C. One unit of activity is defined as the activity necessary to produce 1 μmol of product in 1 min. Kinetic constants were calculated from Hanes-Woolf (*S/V* versus *S*) replots of kinetic data. *K<sub>i</sub>* values were calculated from Dixon (*1/V* versus *I*) plots (8).

Protein was determined by the Bradford dye-binding procedure (1) with ovalbumin as a standard.



**Figure 2.** Fractionation of maize kernel HK activity by Mono Q (0.5 × 5 cm) anion exchange chromatography. Ten mL of filtered crude soluble extract were applied to the column, equilibrated with 10 mM Bicine-NaOH (pH 8.5), 5 mM MgCl<sub>2</sub>, and 1 mM DTT. Activity was eluted by a linear 0 to 300 mM NaCl gradient in equilibration buffer.

## RESULTS

### Analytical Separation of Different Forms of HK

Chromatography of crude maize kernel soluble extract on a Mono Q anion exchanger indicated multiple forms of ATP-dependent hexose kinase activity (Fig. 2), differing in sugar specificity. Three forms of FK and one form of GK could be separated by this procedure. Generally, about 85 to 95% of the HK activity loaded onto the column was recovered. The ratio of FK-1 to FK-2 activities recovered from the column varied from about 1.0 to 1.5.

Chromatography of HKs on a calibrated Ultrogel AcA 44 column resolved two peaks of HK activity. The two major forms of FK (FK-1 and FK-2) and one form of GK (GK-1) eluted in the same fractions with an estimated  $M_r$  of 59,000. A second form of glucokinase (GK-2) eluted with an estimated  $M_r$  of 39,000 (Fig. 3).

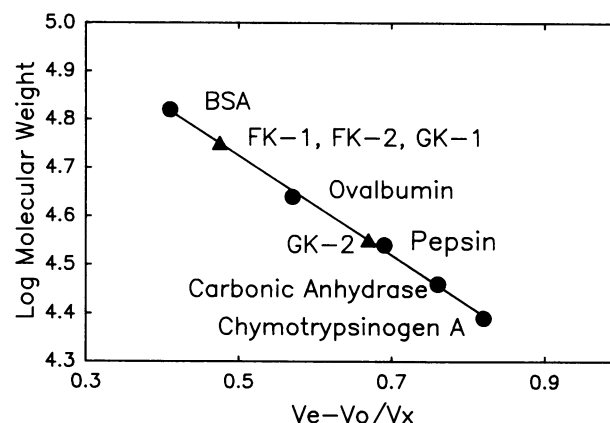
### Preparative Separation of Different Forms of HK

Four forms of HK were separated chromatographically for analysis of catalytic properties. The first step of the separation involved the preparation of a 40 to 80% ammonium sulfate precipitate that was resuspended in buffer and loaded onto an Ultrogel AcA 44 column. The two HK fractions separated by preparative Ultrogel AcA 44 chromatography were analogous to those resolved on the analytical Ultrogel column (Fig. 3), and were designated HK-1 and HK-2. HK-1 contained most of the FK activity as well as GK activity and considerable ketose reductase activity. HK-2 also contained considerable GK activity, but contained much less FK activity.

FK and GK activities were separated on blue-A chromatography, as described in the "Materials and Methods." FK did not bind to blue-A, whereas GK did bind and could be eluted with 1 M NaCl. Ketose reductase also bound to the blue-A column and was eluted in the 1 mM NAD wash. Elution of GK from blue-A columns from HK-1 and HK-2 yielded glucokinase preparations referred to as GK-1 and GK-

2. These preparations were free of interfering activities of ketose reductase and phosphatase (data not shown). FK activity eluted from blue-A chromatography of HK-1 preparations was loaded onto a Mono Q anion exchange column. Blue-A chromatography of HK-2 fractions did not yield enough FK to make further purification steps practical. Chromatography of FKs on Mono Q yielded two forms of FK corresponding to the two major forms of FK seen in Figure 2. These were designated FK-1 and FK-2, in order of their elution. All FK preparations were free of interfering ketose reductase and phosphatase activity. A third form of FK, observed during Mono Q chromatography of crude extracts that eluted prior to FK-1 and FK-2 (Fig. 2), was not recovered by the procedure described and was not analyzed in this study.

The purification of GK-1 represented an increase in specific activity of about 82-fold and about 6% of the activity present in the crude extract was recovered in the GK-1 fraction. The purified GK-2 fraction represented a 90-fold increase in spe-



**Figure 3.** Molecular weight estimation of maize kernel hexose kinases on a calibrated Ultrogel AcA 44 column. The column was equilibrated and eluted with 10 mM Bicine-NaOH (pH 8.5), 5 mM DTT, and 1 mM DTT. The  $M_r$  of standard proteins used were BSA (66,000), ovalbumin (44,000), pepsin (35,000), carbonic anhydrase (29,000), and chymotrypsinogen A (24,500).  $V_0$ , void volume of column;  $V_e$ , elution volume of protein;  $V_x$ , volume occupied by gel, derived from the total bed volume of the column minus  $V_0$  (2).

**Table I.** Sugar Specificity of Four Forms of HKs Isolated from Developing Maize Kernels

Sugars were present at 1.0 mM with 2.5 mM ATP. All assays were coupled with pyruvate kinase, lactate dehydrogenase, and NADH oxidation. Values are derived from averages of three determinations, each from a separate purification.

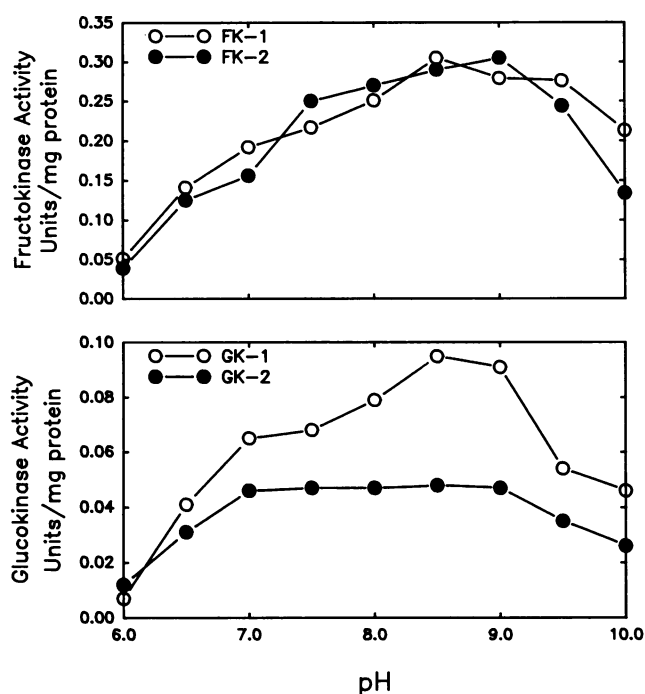
Sugar	Relative Activity			
	GK-1	GK-2	FK-1	FK-2
D-Glucose	100	100	tr <sup>a</sup>	tr
D-Fructose	17	9	100	100
L-Sorbose	0	0	0	tr
D-Mannose	52	32	0	0
Sorbitol	0	0	0	0
L-Glucose	0	0	0	0

<sup>a</sup> Trace.

**Table II.** Nucleotide Triphosphate Specificity of Four Forms of HK Isolated from Developing Corn Kernels

All nucleotide triphosphates were present at 1 mM. GK assays contained 2.5 mM glucose. FK assays contained 0.5 mM fructose. Assays were coupled with NAD reduction through glucose 6-P dehydrogenase. Values are the average of three determinations, each from a separate purification.

Nucleotide Triphosphate	Relative Activity			
	GK-1	GK-2	FK-1	FK-2
ATP	100	100	100	100
UTP	9	14	14	14
CTP	12	5	18	18
GTP	11	7	11	12

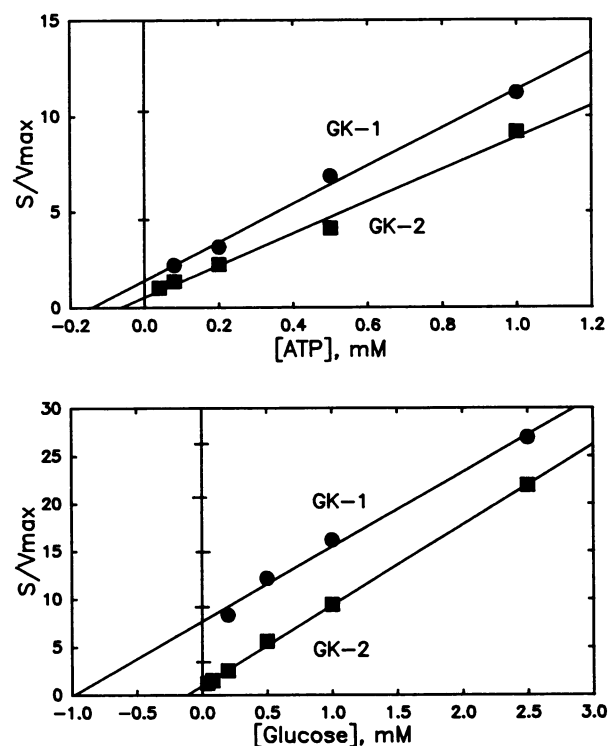
**Figure 4.** The pH response of FKs and GKs isolated from developing maize kernels. Enzymes were assayed in buffer containing 100 mM citrate, 100 mM phosphate, 100 mM Tris, and 100 mM glycine adjusted to the appropriate pH. Activity was detected using a glucose-6-P dehydrogenase coupled assay, where NAD reduction was monitored at  $A_{340}$ .

cific activity with an overall recovery of 7% of activity in the crude extract.

The purification of FK-1 resulted in a 91-fold increase in specific FK activity with an overall yield of about 15% of the activity present in the crude extract. The FK-2 purification resulted in a 135-fold increase in specific activity with a 16% overall yield from the crude extract.

#### Properties of Maize Kernel HK

Sugar specificity was tested for the four HK separated by the above procedure. Both GKs utilized D-glucose most effectively and D-mannose less effectively. These enzymes utilized

**Figure 5.** Hanes-Woolf secondary plots of the substrate saturation response of GK-1 and GK-2 to glucose and ATP. Assays were performed at pH 8.5 and 30°C in the presence of 5 mM  $MgCl_2$ . S, substrate concentration;  $V_{max}$ , units · mg protein<sup>-1</sup>.

D-fructose poorly and would not utilize any other sugar tested (Table I).

FKs utilized D-fructose almost exclusively. Very low rates of D-glucose and L-sorbose utilization were observed (Table I).

All HKs analyzed in this study utilized ATP most effectively as a phosphate donor (Table II). All of these enzymes utilized UTP, CTP, and GTP at less than 20% of the rate of ATP utilization.

All HKs examined here had broad pH optima from 7.5 to 9.5 (Fig. 4). Most forms had optimal activity at pH 8.5 to 9.0, but GK-2 was insensitive to pH over the range of pH 7.0 to 9.0.

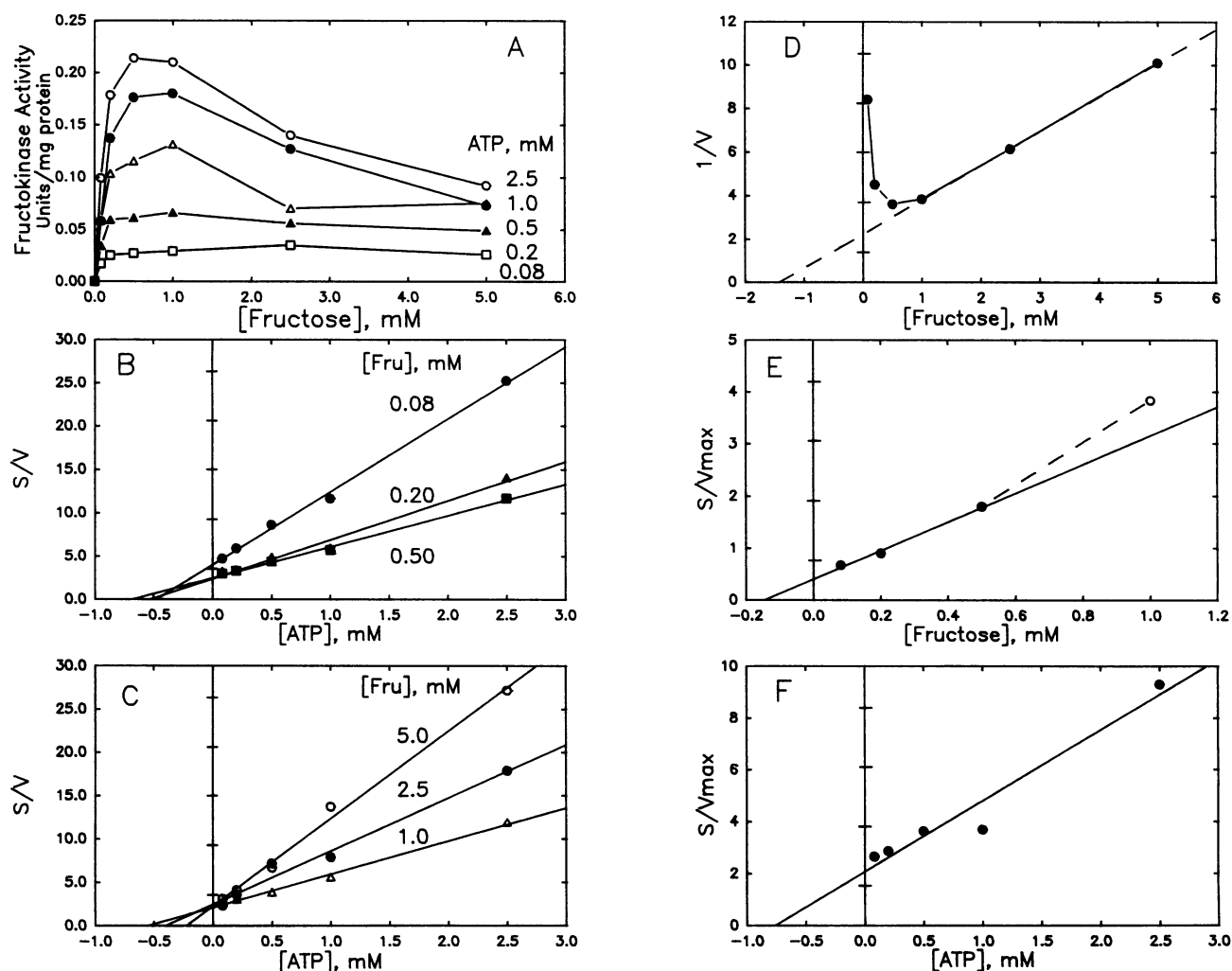
Substrate saturation responses of each HK to ATP and its most effective hexose substrate were determined for all four forms of HK prepared here by generating a series of hexose concentration response curves, each at a different ATP concentration.  $K_m$  values were determined from secondary Hanes-Woolf plots of the kinetic data. Analysis of the two GKs indicated that these two enzymes had very different responses to substrate concentration. GK-1 had much higher  $K_m$  values than GK-2 for both glucose and ATP (Fig. 5, Table III). Both primary (not shown) and secondary replots (Fig. 5) of kinetic data were linear, indicating that both of these enzymes followed Michaelis-Menten kinetics.

Both FK-1 and FK-2 had similar responses to substrate concentrations, thus only the data for FK-1 is shown in Figure 6. Both enzymes had similar  $K_m$  values for both fructose and ATP (Table III). Both enzymes also exhibited substrate inhi-

**Table III. Compiled Kinetic Constants of Four Forms of Maize Kernel HK**

Enzymes were partially purified as described in the "Materials and Methods." Each set of constants is the average of three determinations  $\pm$ SD, each from a separate purification.

HK	$K_m(\text{Hexose})$	$K_m(\text{ATP})$	$K_i(\text{Fructose})$	$V_{\max}$
	$\mu\text{M}$	$\mu\text{M}$	$\text{mM}$	$\text{units/mg protein}$
GK-1	$750 \pm 135$	$182 \pm 4$		0.110
GK-2	$117 \pm 17$	$66 \pm 9$		0.120
FK-1	$148 \pm 26$	$764 \pm 164$	$1.42 \pm 0.32$	0.365
FK-2	$121 \pm 11$	$674 \pm 235$	$2.08 \pm 0.45$	0.540



**Figure 6.** Kinetic plots of FK-1 response to substrate concentration. A, Response of FK-1 activity to increasing fructose concentration at a series of ATP concentrations. FK activity is expressed as units  $\cdot$  mg protein $^{-1}$ . B, Primary Hanes-Woolf plot of FK-1 response to increasing ATP concentration at noninhibiting fructose concentrations. S, substrate (ATP) concentration; V, units  $\cdot$  mg protein $^{-1}$ . C, Primary Hanes-Woolf plot of FK-1 response to ATP concentration at increasing inhibitory fructose concentrations. Units as in B. D, Dixon plot showing substrate inhibition of FK-1 by fructose. V, units  $\cdot$  mg protein $^{-1}$ . E, Secondary Hanes-Woolf plot derived from plot B. Dotted line denotes departure from linearity caused by substrate inhibition. S, substrate (fructose) concentration;  $V_{\max}$ , units  $\cdot$  mg protein $^{-1}$ . F, Secondary Hanes-Woolf plot showing response of FK-1 to ATP concentration in nonlimiting fructose concentrations. S, substrate (ATP) concentration;  $V_{\max}$ , units  $\cdot$  mg protein $^{-1}$ .

bition by fructose (Fig. 6, A, C, and D). Increasing fructose concentration over 0.5 mM caused a decrease in the apparent  $K_m$  (ATP) and a proportional decrease in the  $V_{\max}$  (Fig. 6C). Thus fructose appeared to exhibit uncompetitive substrate inhibition on FK. Both FK-1 and FK-2 had similar  $K_m$  values

for fructose and ATP (Fig. 6, E and F, Table III) and values for  $K_i$  (fructose) (Fig. 6D, Table III).

The  $\text{MgCl}_2$  concentration was held constant at 5 mM for all experiments reported here. Under these conditions, over 99% of the total ATP present in an enzyme was bound by  $\text{Mg}^{2+}$

(14). Thus for practical purposes, the total ATP concentration was equal to the  $\text{Mg} \cdot \text{ATP}^{-2}$  concentration. However when the ATP concentration was varied while the total  $\text{MgCl}_2$  remained constant, the concentration of free  $\text{Mg}^{2+}$  decreased with increasing ATP concentration. Thus in Figures 5 and 6, free  $\text{Mg}^{2+}$  concentration varied from about 4.95 mM (when ATP was 0.05 mM) to about 2.5 mM (when ATP was 2.5 mM). It was determined in separate experiments that the activities of the HK studied here were not affected by changes in free  $\text{Mg}^{2+}$  between 2.0 and 10.0 mM, when  $\text{Mg} \cdot \text{ATP}$  was held constant at 2.5 mM (data not shown). Also, apparent  $K_m$  (ATP) values determined when free  $\text{Mg}^{2+}$  concentration was kept constant at 2.5 mM did not differ significantly from apparent  $K_m$  (ATP) values obtained when total  $\text{MgCl}_2$  was held constant at 5.0 mM (data not shown).

A variety of compounds were tested as possible metabolic effectors of HKs. Sugar phosphates tested including 1 mM Fru-6-P, Fru-1-P, Glc-6-P, Glc-1-P, 6-P-gluconate, Fru-1,6-bisP, 50  $\mu\text{M}$  Glc-1,6-bisP, and 10  $\mu\text{M}$  Fru-2,6-bisP had little effect on any of the HK activities when assayed at pH 8.5 with 2.5 mM ATP and either 2.5 mM glucose for the GKs or 0.5 mM fructose for the FKs (data not shown). GKs were not affected by 2.5 mM fructose, nor were FKs affected by 2.5 mM glucose. Neither 5 mM Pi nor 0.6 mM PPI affected any of the HK activities. All of the HKs were inhibited slightly (10–25%) by either 3 mM phosphoenolpyruvate or 1 mM 2,3-P-glycerate. ADP added to HK assays with sufficient  $\text{MgCl}_2$  to maintain a constant free  $\text{Mg}^{2+}$  concentration of 2.5 mM (14) acted as a strong inhibitor of all of the HKs. ADP at 1 mM inhibited HKs by 20 to 45%, whereas 5 mM ADP inhibited HKs by 60 to 70% (data not shown).

## DISCUSSION

Four forms of hexose-phosphorylating activities have been separated by chromatography. Two forms utilized glucose most effectively and utilized fructose ineffectively. These are classified as GKs (EC 2.7.1.2). Two forms utilized fructose most effectively and would not utilize glucose. These are classified as FKs (EC 2.7.1.4). Additional forms of HK have been detected but were not collected in sufficient quantities to allow characterization.

Earlier results on maize GK from Cox and Dickinson (7) suggested biphasic substrate saturation kinetics for glucose. In this study, two different forms of GK were separated, one with a low  $K_m$  for glucose and ATP and one with a higher  $K_m$  for glucose and ATP. The higher  $K_m$  form presumably would function at higher glucose concentrations. This would suggest that glucose phosphorylating activity is primarily regulated by substrate concentration.

GK-1 studied here resembles a GK isolated from wheat germ (15) with respect to substrate affinities and specificities. The GK-2 described here resembles a GK isolated from pea seeds (18) with respect to substrate specificity,  $K_m$  values and response to glucose 6-P and ADP. Recently, Malaisse-Lagae *et al.* (13) isolated GK from maize embryos and described substrate affinities and an anomeric specificity of this enzyme for  $\alpha$ -D-glucose. This enzyme resembles the GK-2 described in this study, although anomeric specificity was not examined in the present study.

The two forms of FK isolated from developing corn kernels appear to be very similar to each other in nearly all respects. Fructose inhibition has been reported previously for two FKs from mature pea seeds (3, 4, 17) and for soybean nodule FK (5). The FKs described in this study appear to be similar to those plant FKs described earlier. Substrate inhibition of FK by fructose may be a regulatory mechanism for this enzyme.

Copeland *et al.* (4) analyzed substrate inhibition in a pea seed FK and found inhibition by both fructose and ATP. At the ATP concentrations tested in this study, no inhibition by ATP was found. However, ATP inhibition was apparent in the study of Turner *et al.* (17) only at pH 6.6, or at pH 8.2 over 6 mM ATP, conditions that were not tested in this study. Thus, it is not clear if the kinetic mechanism suggested by Copeland *et al.* (4) is consistent with the results presented here.

The FKs examined in this study are fairly specific for ATP as the phosphate donor. It appears that the enzymes isolated here are not responsible for observed UTP-dependent fructokinase activity that has been observed in previous studies on developing corn kernels (9, 10). An additional form of FK that utilized UTP more effectively than ATP has been separated in this laboratory (data not shown), but sufficient quantities for characterization are not yet available.

The intracellular localization of the HKs was not addressed in this study. In a genetic study by Wendel *et al.* (19), HK activity appeared only in cytosolic preparations and not in mitochondria or plastid preparations from germinating corn coleoptiles. Echeverria *et al.* (11) also determined that HK activity was not associated with amyloplast preparations derived from developing corn endosperm.

It is apparent that HK activity measured in crude corn kernel extracts is comprised of a number of different forms of specific GKs and FKs. Different forms of HK may have specialized functions in the kernel. The determination of the intracellular localization and the distribution of HK forms within the kernel will provide important information as to the functional relationships of these enzymes.

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